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(54) Antibodies specific to endotoxins.

(57) The invention is concerned with the problem of gram-negative bacteremia and endotoxemia. The invention provides a composition comprising one or more antibodies which are specific to one or more endotoxins, especially those produced by gram-negative bacteria, in admixture with a carrier medium. The carrier medium may be animal or human serum, milk or a synthetic material. The invention also provides a method of producing a composition comprising an antibody or a mixture of antibodies specific to endotoxin(s), including the steps of inoculating a suitable animal with an antigen or mixture of antigens capable of triggering the appropriate immunological response in the animal, and removing a body fluid comprising the resulting antibody or mixture of antibodies.

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ANTIBODIES SPECIFIC TO ENDOTOXINS

This invention relates to compositions of antibodies specific to endotoxins.

Gram negative bacteremia and endotoxemia is an important medical problem carrying a mortality of 30-80 % and causes millions of deaths in humans and animals world wide every year. Conventional antibiotic therapy is becoming less successful with time because antibiotics kill bacteria but do not at all reduce the amount of the extremely potent and stable endotoxin released from gram negative bacterial cells. This endotoxin is still active upon death of bacteria and can continue to cause shock and death. In addition under certain conditions endotoxin may leak out of the intestine where it is always present and enter the systemic blood causing shock and death. A notable result of this is the very high mortality of farm animals having gram negative sepsis.

Endotoxin is known to be present in the intestines and under certain conditions of stress may leak out through the intestinal wall and enter the systemic blood supply, overwhelming the liver's detoxifying capacity and causing shock and death. In these cases there may not have been a stage of bacteremia prior to endotoxemia.

A means of inactivating or reducing the free concentration of endotoxins is by administering appropriate antibodies specific to endotoxins.

In the prior art it is known to immunise cows with the "pili" of gram negative bacteria to produce milk rich in "antipili" antibodies. Such antibodies prevent the attachment or adhesion of gram negative bacteria to the mucosal surface in the gut and in this manner reduce bacterial infection.

It is an object of the invention to provide a composition containing a high titre of antibodies which are specific to endotoxins produced by gram negative bacteria. It is a further object of the invention to incorporate such antibodies in products for human and veterinary use.

The present invention provides a composition comprising one or more antibodies which is or are specific to one or more endotoxin(s), in admixture with a carrier medium. The carrier medium preferably comprises serum obtained by suitable treatment of the blood of animals or it may be synthesised from suitable materials, for example it may be physiological saline. The composition may be in a form suitable for oral or parenteral administration to animals and/or humans, or for incorporation in milk or a milk derivative.

The antibody or antibodies may preferably be

specific to endotoxin(s) produced by gram negative bacteria of the following genus: Salmonella, Eschericia, Pseudomonas, Shigella, Serratia, Vibrio and Yersinia. It will be appreciated that the antibody or antibodies may react with the endotoxin-producing organism itself, in addition to "free" endotoxin(s) produced by the organism. Such binding to the endotoxin coat of living bacteria may activate complement which in turn may cause lysis and death of the bacteria.

According to a further aspect, the invention provides a method of producing a composition comprising one or more antibodies specific to endotoxin(s), which process includes the steps of inoculating a suitable animal with an antigen or an antigen mixture capable of triggering the appropriate immunological response in the animal and removing a body fluid containing the resulting antibody or mixture of antibodies. The body fluid may be worked up in a known manner to give a preparation suitable for therapeutic and/or prophylactic use in a human and/or animal. Such methods are well known in the art.

The antigen or antigen mixture comprising endotoxin(s) produced by gram negative bacteria is generally injected into an animal to trigger an immunological response to produce the corresponding

antibodies. The endotoxin(s) may be sensitised in accordance with accepted laboratory methods and injected in suitable animals under controlled conditions. An adjuvant, for example Freud's Complete Adjuvant, is preferably present in the composition to be injected.

Antibodies prepared in this manner may be type specific e.g. to endotoxins produced by Salmonella enteriditis, or may have a more general specificity, for example to endotoxins produced by many or all gram negative bacteria, or may be a mixture of the above types of antibodies.

The animal to be used for the production of the desired antibodies may be a farm animal, for example a bovine, equine, porcine, ovine, canine or feline animal. The immunisation is, for example, subcutaneous, and the immunisation is generally repeated as often as necessary to maintain a high level of antibodies in the blood of the immunised animal, which may be used subsequently for the preparation in known manner of the desired antiserum for therapeutic or prophylactic use in the same species of animal or in another species. In the latter case, the serum is preferably subjected to a treatment which generally includes reacting the serum preparation with the blood, blood cells, blood fraction(s) or another tissue

preparation of animals of the species to be treated, then removing the added blood or other tissue component(s), for example by centrifugation and/or column chromatography and/or any other procedure.

An antibody composition of the invention preferably comprises a total of not less than 100 micrograms of antibodies per millilitre.

An antibody composition of the invention may be in unit dose or multidose containers, and may be in lyophilised form, for reconstitution before use.

According to one embodiment of this invention, the antibody or antibodies specific to the endotoxin(s) may be incorporated in milk or a byproduct thereof. This is particularly useful where antibodies specific to endotoxins are not present naturally in adequate levels in human colostrum and breast milk. Pregnant or lactating cows, mares, ewes, bitches or cats may be immunised with endotoxins to provoke the production of antibodies specific to endotoxins, for example as described above. The milk obtained from such animals contains a high concentration of anti-endotoxin antibodies. This milk or products prepared from this milk may be given to human and animals neonates and children whose mothers' milk is deficient in these antibodies and/or who may benefit from the resulting prophylaxis.

The following Examples illustrate the invention.

Example 1

Cows and other farm animals were immunised by the sub-cutaneous injection of a solution of endotoxins obtained from about 12, for example 11, different bacterial strains and species, which solution was emulsified with an equal volume of Freud's Complete Adjuvant.

The mixture of endotoxins injected above were those obtained from Salmonella enteriditis, Salmonella typhosa, Salmonella typhimurium, Salmonella abortus equii, Eschericia coli 026:B6, Eschericia coli 0128:B12, Eschericia coli 0127:B8, Eschericia coli 055:B5, Salmonella minnesota, Serratia marcescens, and Klebsiella pneumoniae, and, optionally, Shigella flexneri and/or E. coli 0111:B4.

The above composition comprising endotoxins with Freud's Complete Adjuvant was injected as often as required to maintain a high antibody level in milk and/or serum.

a) Milk obtained from the suitably immunised animals that were pregnant or lactating was further processed in the same way as normal milk. The resulting milk, and other products obtained therefrom, was suitable for administering to human and animal neonates and young.

b) Serum which contains a high titre of antibodies specific to the above endotoxins was prepared by

bleeding the immunised animal and working up the blood by a method known for the production of an antiserum from blood.

Other bacterial species or strains may be substituted for any one or more of those named above.

Example 2

1. There were obtained compositions of equine, bovine, sheep, canine, feline and porcine serum comprising antibodies which react with and bind endotoxins prepared from the following bacterial sources:

Salmonella typhosa

Salmonella typhimurium

Salmonella abortus equii

Shigella flexneri

Eschericia coli 055:B5

Eschericia coli 0127:B8

Eschericia coli 0128:B12

Eschericia coli 026:B6

Eschericia coli 0111:B4

Salmonella minnesota

Serratia marcescens

Klebsiella pneumoniae

The compositions were prepared as described in Example 1.

2. The total concentration in the composition of all the above anti-endotoxins together was not less than 100 micrograms per millilitre and there was a minimum

concentration of 1 microgram per millilitre of at least ten of the about twelve species or strains.

3. The preparation had the ability to kill Klebsiella pneumoniae bacteria in the presence of complement and serum.

4. a) Serum prepared by suitably inoculating a horse was used therapeutically or prophylactically on other horses.

b) Serum obtained by suitably inoculating a horse was suitably treated to render it fit to use therapeutically or prophylactically on another species of animal, e.g. a cow, sheep, pig, cat or dog.

5. The treatment required in 4b above included reacting the serum preparation with the blood, blood cells, blood fraction(s), or another tissue preparation of animals of the species to be treated, then removing the added blood or other tissue component(s) by centrifugation and/or column chromatography, although any other suitable procedure could be used instead or in addition.

Other strains or species of bacteria may be used instead of or in addition to those listed above, and one or more of the listed sources may be omitted if desired.

Other animals e.g. farm animals may be used for the preparation of serum for therapeutic or

prophylactic use in the same or another species of animal, for example as described in 4 and 5 above for horses.

Claims

1. A composition comprising one or more antibodies which are specific to one or more endotoxin(s), in admixture with a carrier medium.

2. A composition according to claim 1 in which the antibody or antibodies is or are specific to endotoxin(s) produced by one or more species or strains of bacteria.

3. A composition according to claim 1 or claim 2 in which the antibody or antibodies is or are specific to endotoxin(s) produced by gram negative bacteria of one or more of the following species: Salmonella, Shigella, Eschericia, Pseudomonas, Serratia, Vibrio or Yersinia.

4. A composition according to claim 1 or claim 2, wherein the antibodies are specific to endotoxins obtained from Salmonella enteriditis, Salmonella typhosa, Salmonella typhimurium, Salmonella abortus equii, Eschericia coli 026:B6, Eschericia coli 0128:B12, Eschericia coli 0127:B8, Eschericia coli 055:B5, Salmonella minnesota, Serratia marcescens, and Klebsiella pneumoniae, and, optionally, Shigella flexneri and/or E. coli 0111:B4;or from Salmonella typhosa, Salmonella typhimurium, Salmonella abortus equii, Shigella flexneri, Eschericia coli 055:B5, Eschericia coli 0127:B8, Eschericia coli 0128:B12,

Eschericia coli 026:B6, Eschericia coli 0111:B4, Salmonella minnesota, Serratia marcescens and Klebsiella pneumoniae.

5. A composition according to any one of claims 1 to 4, in which the carrier medium comprises animal or human serum, or one or more synthetic material(s), or milk or a derivative thereof.

6. A composition according to any one of claims 1 to 5, suitable for parenteral or oral administration to a human or an animal.

7. A method of producing a composition comprising an antibody or a mixture of antibodies specific to endotoxin(s), including the steps of inoculating a suitable animal with an antigen or mixture of antigens capable of triggering the appropriate immunological response in the animal, and removing a body fluid comprising the resulting antibody or mixture of antibodies.

8. A method of producing an antibody or a mixture of antibodies according to claim 7, which includes the steps of injecting an antigen or a mixture of antigens comprising endotoxin(s) produced by one or more gram negative bacteria into an animal, and withdrawing blood from the animal to obtain the resulting antibody or antibody mixture.

9. A method of producing an antibody or a

mixture of antibodies according to claim 7, which includes the step of inoculating a pregnant or lactating mammal with one or more endotoxin(s), allowing the animal to develop an immunological response to the endotoxin, and milking the mammal to obtain milk comprising the antibody or mixture of antibodies specific to the endotoxin(s).

10. A method according to any one of claims 7 to 9, wherein the antigen or mixture of antigens is selected from endotoxin(s) produced by gram negative bacteria of one or more of the following species: Salmonella, Shigella Eschericia, Pseudomonas, Serratia, Vibrio or Yersinia.

11. A method according to any one of claims 7 to 9, wherein the antigen mixture comprises endotoxins obtained from Salmonella enteriditis, Salmonella typhosa, Salmonella typhimurium, Salmonella abortus equii, Eschericia coli 026:B6, Eschericia coli 0128:B12, Eschericia coli 0127:B8, Eschericia coli 055:B5, Salmonella minnesota, Serratia marcescens, and Klebsiella pneumoniae, and, optionally, Shigella flexneri and/or E. coli 0111:B4; or from Salmonella typhosa, Salmonella typhimurium, Salmonella abortus equii, Shigella flexneri, Eschericia coli 055:B5 Eschericia coli 0127:B8, Eschericia coli 0128:B12 Eschericia coli 026:B6, Eschericia coli 0111:B4,

Salmonella minnesota, Serratia marcescens and Klebsiella pneumoniae.

12. An antibody or antibody mixture whenever produced by a method according to any one of claims 7 to 11.

13. Milk or serum whenever obtained by a method according to any one of claims 7 to 11.